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Research Article

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Nanoleakage of apical sealing using a calcium silicate-based sealer according to canal drying methods

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Author Contributions
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ABSTRACT

Objectives: This study investigated the nanoleakage of root canal obturations using calcium silicate-based sealer according to different drying methods.
Materials and Methods: Fifty-two extracted mandibular premolars with a single root canal and straight root were selected for this study. After canal preparation with a nickel-titanium rotary file system, the specimens were randomly divided into 4 groups according to canal drying methods (1: complete drying, 2: blot drying/distilled water, 3: blot drying/NaOCl, 4: aspiration only). The root canals were obturated using a single-cone filling technique with a calcium silicate–based sealer. Nanoleakage was evaluated using a nanoflow device after 24 hours, 1 week, and 1 month. Data were collected twice per second at the nanoscale and measured in nanoliters per second. Data were statistically analyzed using the Kruskal-Wallis and Mann–Whitney *U*-tests ($p < 0.05$).
Results: The mean flow rate measured after 24 hours showed the highest value among the time periods in all groups. However, the difference in the flow rate between 1 week and 1 month was not significant. The mean flow rate of the complete drying group was the highest at all time points. After 1 month, the mean flow rate in the blot drying group and the aspiration group was not significantly different.
Conclusions: Within the limitations of this study, the canal drying method had a significant effect on leakage and sealing ability in root canal obturations using a calcium silicate-based sealer. Thus, a proper drying procedure is critical in endodontic treatment.

Keywords: Bioceramic sealer; Calcium silicate-based sealer; Canal drying methods; Canal moisture condition; Final irrigant

INTRODUCTION

The purpose of endodontic treatment is to prevent re-infection by blocking all possible potential pathways that cause leakage between the periodontal tissue and the root canal, and it has been reported that a significant number of endodontic treatment failures occur due to leaks at the apical end [1,2]. Therefore, for successful endodontic treatment, it is important to completely seal the entire root canal 3-dimensionally through the root canal filling to minimize leakage at the apical end.

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Traditionally, gutta-percha, which is the most commonly used root canal filling material, does not bind itself to dentin and leaves a space between the unevenly shaped root canal wall. A root canal filling sealer is needed to fill the empty space between the root canal wall and the gutta-percha, while allowing gutta-percha to adhere to the root canal wall [3,4].

Sealers require properties such as insolubility from tissue fluid, biocompatibility, appropriate flowability, antibacterial properties, and adhesiveness [5,6]. However, various types of sealers have been developed, and there is no single sealer that satisfies all these requirements.

AH Plus, an epoxy resin-based sealer, has been used as the gold standard due to its characteristics such as strong adhesion to dentin, appropriate radiopacity, flowability, volume stability, low solubility, and high wash-out resistance [7-9]. There are also disadvantages, such as an initial inflammatory reaction until complete polymerization [10-12].

In recent years, calcium silicate-based sealers, also called bioceramic sealers, have attracted attention [13]. They are alkaline and release calcium ions to form an apatite layer [10,14]. Thus, their bioactivity and biocompatibility can be said to be important features compared to conventional sealers [15]. They also have the advantage of a fast setting time, not being easily washed off, not discoloring teeth, appropriate impermeability, and good biocompatibility [16]. Additionally, they are convenient for clinicians to use as they are provided in a sealed syringe in a pre-mixed state, enabling them to be applied directly inside the root canal without a mixing process.

However, the most important difference between this new bio-ceramic sealers and resin-based sealers is that, unlike conventional resin-based sealers, which require the inside of the root canal to be dried as much as possible before root canal filling, the new sealers have hydrophilicity that allows setting even when moisture exists in the root canal [17,18]. Moisture plays an important role in the setting of the sealer; thus, the method used to dry the root canal immediately before root canal filling can directly affect the quality of root canal filling [13]. However, the perception of appropriate moisture conditions differs among clinicians. Furthermore, root canal drying is usually done using a paper point, and depending on the exact method used, the remaining moisture condition also varies [19]. Manufacturers also recommend leaving adequate moisture when using the product, without providing clear clinical information about the ideal residual water content. Several studies have reported that residual moisture in the root canal affects the sealing ability of sealers. Despite this, there is still a lack of research on the optimal root canal drying method to achieve the highest quality root canal filling [20-24].

Various methods, including the dye penetration test, bacteria leakage test, radioisotope technique, and fluid filtration technique, have traditionally been employed to assess leakage at the proximal end [25-31]. Among these, the fluid filtration technique is commonly used to measure leakage at the root end. This technique gauges the degree of leakage by detecting the movement of an air bubble in the capillary tubule. It provides quantitative data on leakage within the root canal and allows for repeated measurements over time without causing damage to the specimen [32]. Traditional fluid filtration methods measure leakage in microliters per minute (mL/min) by introducing water into the root canal's empty space under a pressure of 0.1 atm at a specific time. However, the recently introduced Nanoflow sub-nanoliter scaled fluid flow measuring device (NFMD) (IB Systems, Seoul, Korea) offers more precise and reliable results (**Figure 1**). It detects bubble movement through an optical sensor with a resolution of 0.196 nL or more in real time, rather than at a specific point in time [33]. The aim of this study is to compare the root end sealing ability of different

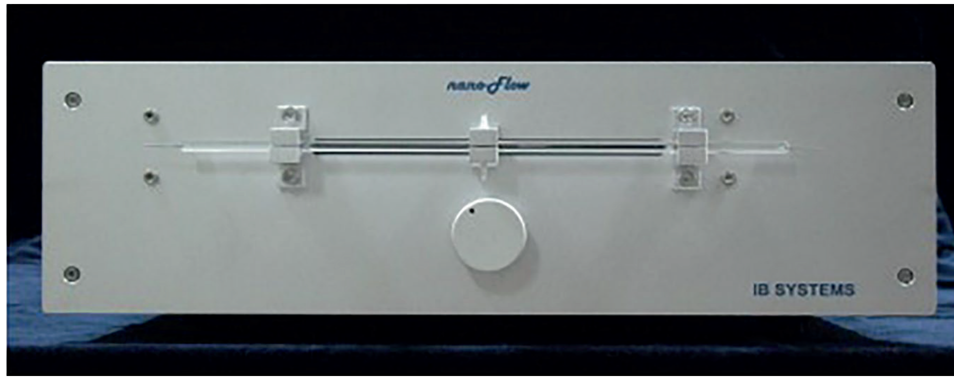

Figure 1. IB SYSTEMS Nanoflow measuring device used to measure nanoleakage.

clinically applicable root canal drying methods using the NFMD in root canals filled with calcium silicate-based sealers. Additionally, the study will compare the sealing ability according to the type of final irrigant used.

MATERIALS AND METHODS

This study was conducted with the approval of the Institutional Ethics Committee of Gangneung-Wonju National University Dental Hospital (IRB 2022-A011). We calculated the sample size assuming a significance level of 0.05, and a power of 0.95 in G*power software version 3.1 (University of Düsseldorf, Düsseldorf, Germany).

Tooth selection

This study was performed on 52 mandibular premolars extracted for orthodontic purposes at Gangneung-Wonju National University Dental Hospital. The tooth selection criteria were as follows:

1. Inclusion criteria
   - Mandibular premolars with straight roots and 1 canal
   - Teeth with complete apexes

2. Exclusion criteria
   - Teeth with cracks, caries, fractures, and canal calcification
   - Teeth with external resorption or macroscopically immature apexes

Research materials

The root canal filling sealer used in this study was AH Plus Bioceramic sealer (Dentsply Sirona, Charlotte, NC, USA), the components of which are zirconium dioxide, tricalcium silicate, dimethyl sulfoxide, lithium carbonate, and thickening agents. Composition and setting time of the material is provided by the manufacturer (Dentsply Sirona) as shown in **Table 1**.

Table 1. The tested calcium silicate-based sealer

Material	Manufacturer	Composition	Setting time
AH Plus Bioceramic sealer	Dentsply Sirona, Charlotte, NC, USA	Zirconium dioxide Tricalcium silicate Dimethyl sulfoxide Lithium carbonate Thickening agents	2–4 hr

Specimen preparation

After extraction, all teeth were stored in Hank's Balanced Salt Solution and then soaked in a 2.5% NaOCl solution at room temperature for 24 hours to eliminate organic residues. The root surface was subsequently cleaned using an ultrasonic instrument and stored in a physiological saline solution prior to the experiment. Once the crown was detached from the cemento-enamel junction, a #10 K-file (Dentsply Sirona, York, PA, USA) was inserted into the root canal until it was visible in the apical foramen. The working length was then determined by subtracting 0.5 mm from this length. To ensure uniformity, all roots were adjusted to have the same working length of 10 mm. Each canal was shaped to accommodate a F4 ProTaper Universal rotary file (Dentsply Maillefer, Ballaigues, Switzerland). During the root canal preparation, 1.0 mL of 2.5% sodium hypochlorite (NaOCl) was used to rinse the root canal after each file use. Upon completion of the root canal preparation, 1.0 mL of 2.5% NaOCl and 1.0 mL of 17% EDTA were sequentially applied for 1 minute to thoroughly remove any residues within the root canal. This was followed by a final rinse with 5.0 mL of distilled water (**Figure 2**). All prepared samples were randomly allocated to 4 experimental groups of 12, along with 2 positive and negative controls. Root canal drying for each experimental group was then carried out as follows (**Table 2**).

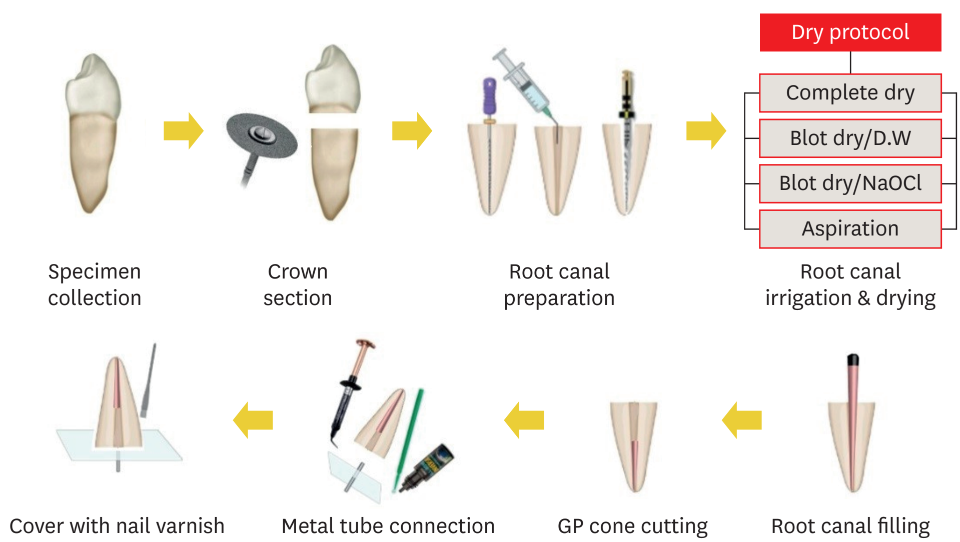

Figure 2. Schematic diagram of specimen preparation.
GP, gutta-percha.

Table 2. Experimental setup and groups

Groups	No.	Drying method
Experimental		
1 Complete drying	12	Complete drying with alcohol and storage at room temperature for 24 hr
2 Blot drying/distilled water	12	Blot drying with 2 paper points after final irrigation using distilled water
3 Blot drying/NaOCl	12	Blot drying with 2 paper points after final irrigation using NaOCl
4 Aspiration only	12	Aspiration with a 27-gauge needle
Control		
5 Positive	2	The root canal was not obturated
6 Negative	2	The entire surface of the root canal was covered with nail varnish after obturation

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1. Group 1: complete drying
After the final root canal irrigation, 1.0 mL of 95% ethanol solution was applied to the root canal using syringe with the same method as NaOCl irrigation, and the needle tip of the syringe (Blue Endo Irrigation Tip, Jiangxi Glance Medical Equipment Co., Jiangxi, China) was placed 3 mm above the apical foramen and aspirated. The root canal was then dried with three or four 40 size paper points with a taper of .04 until it was confirmed that the last paper point appeared completely dry, and then stored at room temperature for 24 hours.

2. Group 2: blot drying/distilled water
After the final root canal irrigation, two size 40 paper points with a taper of .04 were inserted up to the working length and applied for 1 second.

3. Group 3: blot drying/NaOCl
After the final root canal irrigation, 1.0 mL of NaOCl was applied to the root canal, and the needle tip of the syringe was placed 3 mm above the apical foramen and aspirated. Next, two 40 size paper points with a taper of .04 were inserted up to the working length and applied for 1 second.

4. Group 4: aspiration only
After the final root canal irrigation, only aspiration was performed after placing the needle tip of the syringe 3 mm above the foramen.

After canal drying, the root canals were filled using a 40 size gutta-percha cone with .04 taper, along with AH Plus Bioceramic sealer. The upper portion of the gutta-percha cone was then cut, leaving 5 mm of the apical end, using the Super Endo Alpha (B&L Biotech, Ansan, Korea). To prevent unnecessary nanoleakage, nail varnish was applied to the entire root surface, excluding the foramen area, for all specimens. The prepared tooth roots were then wrapped in 4 × 4 gauze that had been soaked in distilled water, and stored in an environment with a temperature of 37°C and 100% humidity. In the positive control group, the root canal was left unfilled. Conversely, in the negative control group, nail varnish was applied to the entire root surface, including the apical foramen area, after the root canal was filled, following the same procedure as in experimental group 2.

Measurement of nanoleakage

The nanoleakage of each test tooth was quantified using an NFMD. To link the specimen with the device, a 20-gauge metal tube was inserted into the root canal of each tooth and secured. A hole was created in the center of a 10 × 10 mm OHP film using a tapered fissure bur (TC-11F Dia-burs, MANI Inc., Tochigi, Japan). This film was positioned at the junction of the tooth root and the metal tube. Subsequently, Single Bond Universal (3M ESPE, St. Paul, MN, USA) and G-aenial Universal Flo (GC Corp., Tokyo, Japan) were applied to seal the junction. The assembly was then connected to a silicone tube, which in turn was linked to the glass capillary of the NFMD filled with distilled water. An air bubble was introduced into the glass capillary to enable an optical sensor to detect movement. The quantities of displaced distilled water, as detected by the movement of the air bubbles, were automatically recorded by the computer software twice per second in real-time (**Figure 3**). The nanoleakage flow rate was measured for 300 seconds, following a 300-second stabilization period for the fluid flow at 21°C and 50 cm air pressure. A stable nanoleakage value was achieved after 5 minutes (300 seconds). The nanoleakage measurement was conducted 3 times in total, at 24 hours, 1 week, and 1 month after the sample preparation (**Figure 4**).

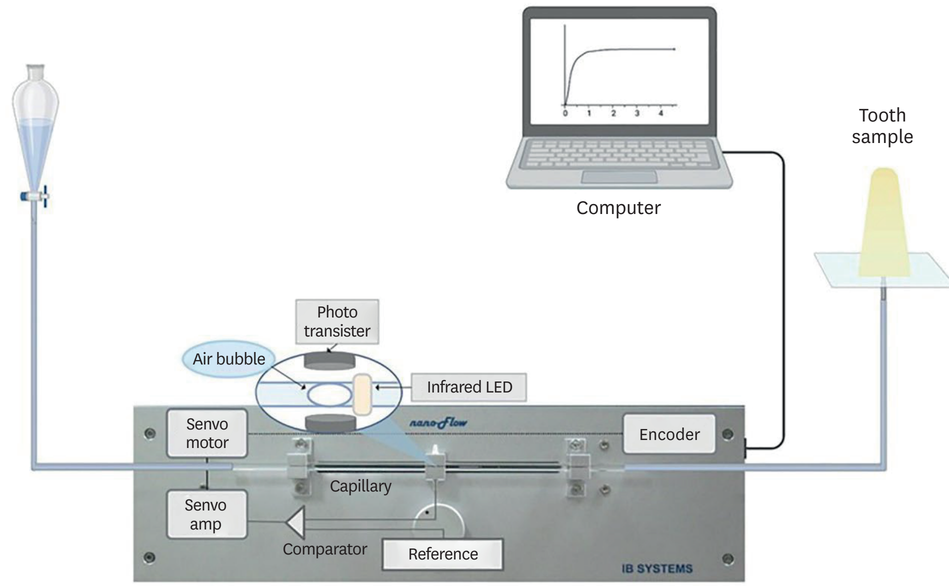

Figure 3. Schematic diagram of the Nanoflow sub-nanoliter-scale dentinal tubular fluid flow measurement device connected to a specimen.

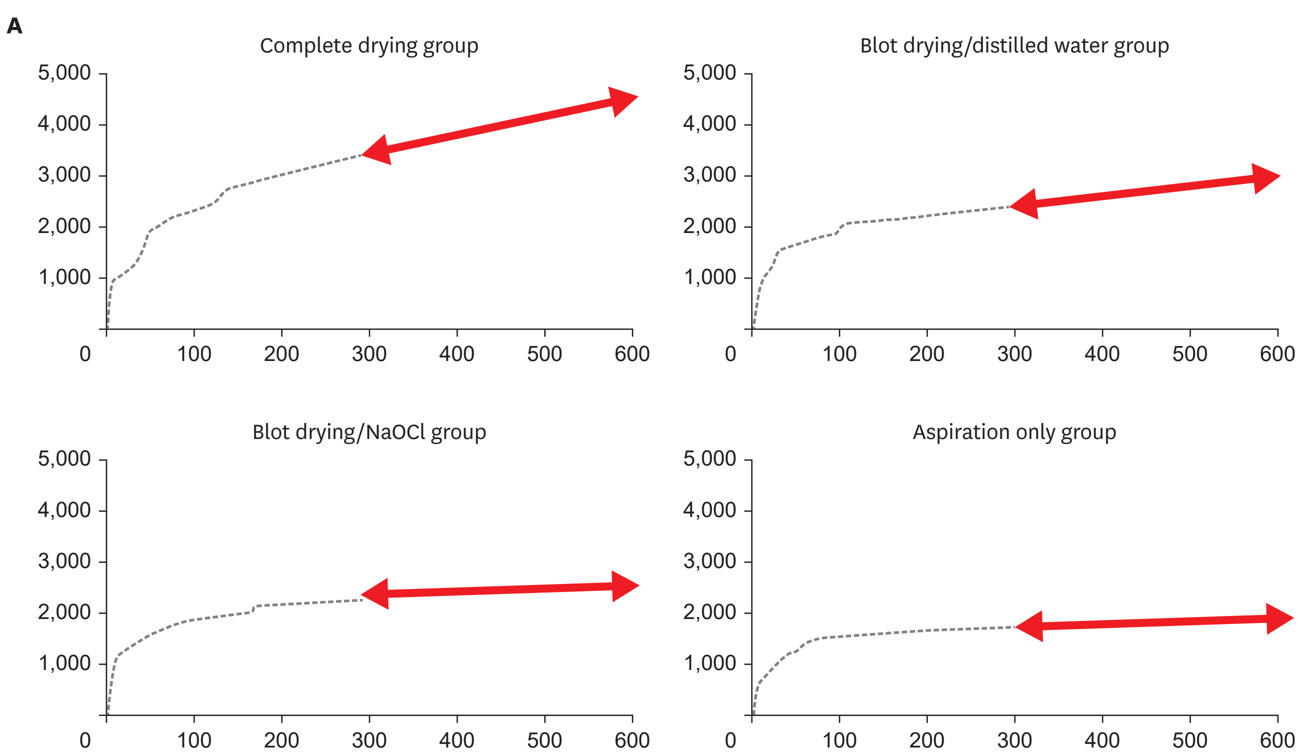

Figure 4. Representative graphs of fluid flow (nL) after (A) 24 hours, (B) 1 week, and (C) 1 month. Leakage was measured as the flow rate (the slope of the graph) after fluid flow stabilization (black arrows).

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Statistical analysis

Statistical analysis was performed using the Kruskal-Wallis test and the Mann-Whitney *U* test, with SPSS version 26 (IBM Corp., Armonk, NY, USA) at the 95% significance level. A run test was performed to verify the randomness of the sample selection. The results were significant, indicating that the sample was indeed randomly selected. To determine if

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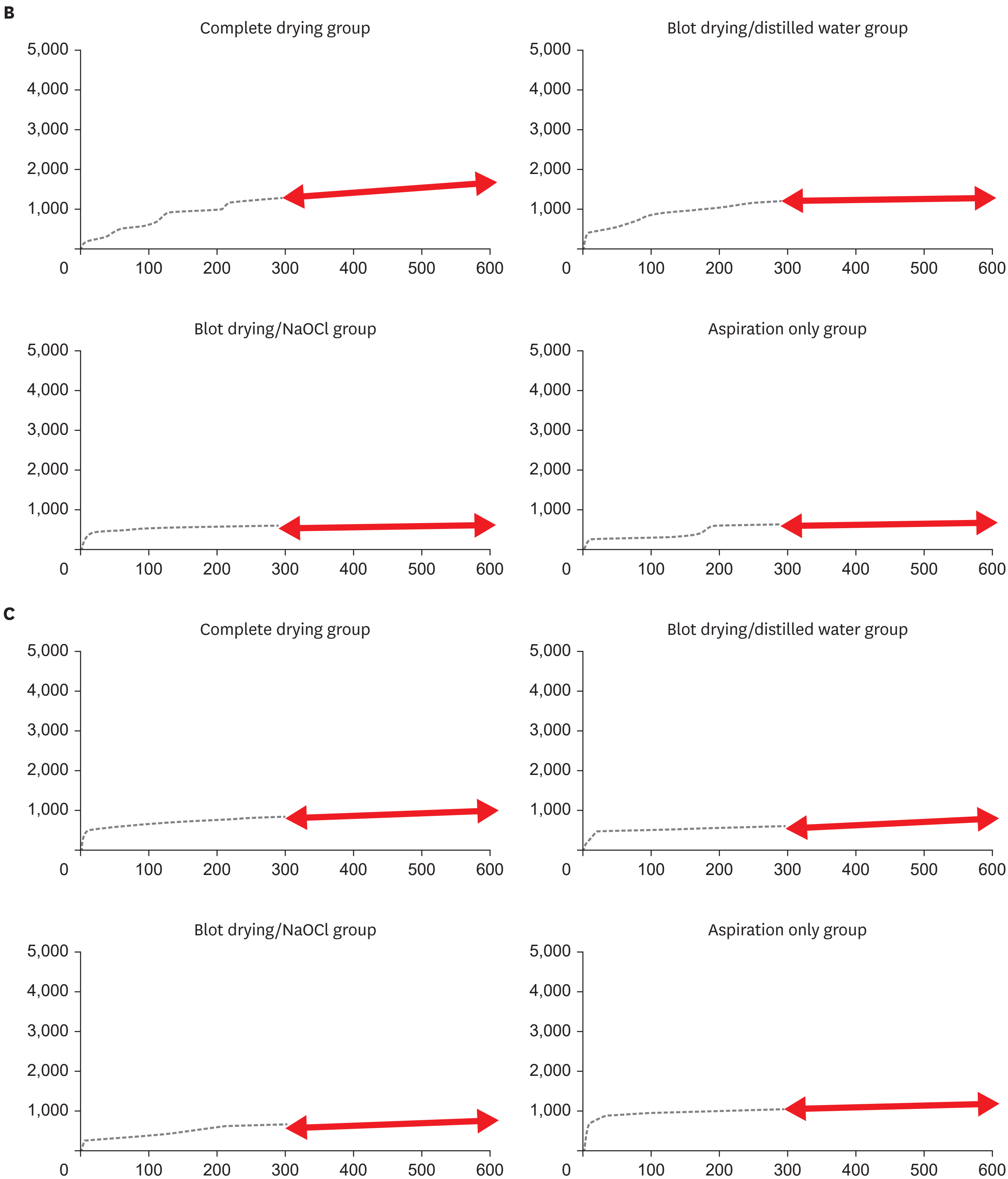

Figure 4. (Continued) Representative graphs of fluid flow (nL) after (A) 24 hours, (B) 1 week, and (C) 1 month. Leakage was measured as the flow rate (the slope of the graph) after fluid flow stabilization (black arrows).

there were significant differences among the experimental groups, a single-factor analysis of variance (repeated-measured analysis of variance, MANOVA) was carried out. In the

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multivariate analysis, Pillai's Trace and Wilks' Lambda were utilized, as sphericity was not assumed. The Tukey HSD test was also conducted. As the measured values did not meet the criteria for normality, a non-parametric test was employed. The Kruskal-Wallis test was used to ascertain if there were significant differences among the experimental groups over the measurement period. The Mann-Whitney *U* test was then used to identify which experimental group and measurement period exhibited differences.

RESULTS

The values for the accumulated amount of nanoleakage (nL) and nanoleakage flow rate per second (nL/s) was obtained, and the results were analyzed using flow rate values obtained between 300 and 600 seconds.

Intragroup comparisons

Table 3 presents the nanoleakage flow rate for each group at various time points. In every group, the nanoleakage flow rate recorded after 24 hours was significantly greater than the rates observed after 1 week and 1 month. Conversely, the differences in flow rates between the 1-week and 1-month time points were not statistically significant across all groups.

Intergroup comparisons

Significant differences were observed across all time points when comparing Group 1 to the remaining experimental groups (**Figure 5**). Specifically, the group where the root canal was thoroughly dried demonstrated a significantly elevated nanoleakage flow rate at the 24-hour, 1-week, and 1-month marks, compared to the root canals dried using alternative methods.

When comparing the group where 2 paper points were applied to the root canal for 1 second each and the root canal was subsequently dried in the same manner with the group where the final cleaning solution was either distilled water or NaOCl, a significant difference was found in the nanoleakage flow rate after 24 hours and 1 week. However, after 1 month, the difference between the 2 groups was not significant.

In a comparison of the group where the root canal was dried using 2 paper points following the final irrigation with NaOCl, to the group where the root canal was dried solely through aspiration, no significant difference was observed at any time point.

Finally, upon a comparison of the groups after 1 month, no significant difference was observed between them, with the exception of the group where the root canal had been completely dried.

Table 3. Intra- and inter-group comparison of nanoleakage according to each time point and canal drying methods

Group	No.	Time		
		24H	1W	1M
Complete drying	12	3.41 ± 2.07^{Aa}	1.41 ± 0.52^{Ba}	0.71 ± 0.25^{Ba}
Blot drying/distilled water	12	1.82 ± 0.52^{Ab}	0.15 ± 0.07^{Bc}	0.43 ± 0.15^{Bb}
Blot drying/NaOCl	12	0.69 ± 0.43^{Ac}	0.29 ± 0.21^{Bb}	0.33 ± 0.13^{Bb}
Aspiration only	12	0.48 ± 0.26^{Ac}	0.28 ± 0.19^{Bbc}	0.35 ± 0.25^{Bb}

Values are presented as mean ± standard deviation.
The mean values that are followed by the same superscript uppercase letter in the rows indicate no statistically significant difference between time points. In the columns, the same superscript lowercase letter indicates no statistically significant difference among experimental groups.

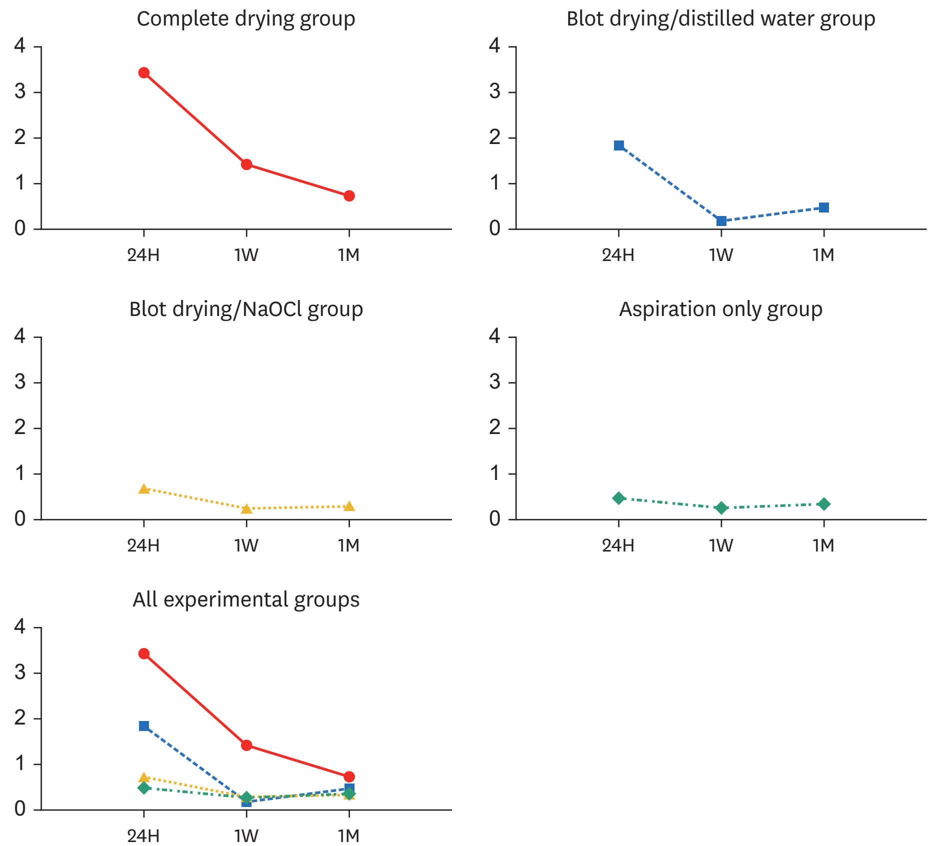

Figure 5. Line charts of the mean nanoflow in the experimental groups.

The positive control group consistently exhibited very high nanoleakage at each measurement interval, rendering it impossible to measure for a duration of 600 seconds. Conversely, the negative control group consistently displayed a nanoleakage value of 0 throughout all measurement periods.

DISCUSSION

This study is the first to compare the sealing capacity at the apical end, based on the root canal drying method and the type of final irrigation solution used in root canals filled with a calcium silicate-based sealer, by measuring nanoleakage. A newly developed high-performance instrument, the NFMD, was employed to measure nanoleakage. This allowed the assessment of root canal nanoleakage at the nanoliter level in real-time.

Leakage experiments have been conducted to evaluate the sealing ability of the apical end through leakage tests. However, there are limitations to the quantitative analysis that can be achieved by comparing the initial and final values in each specimen [34]. Many studies have utilized fluid filtration instruments, with the method developed by Pashley (Flodec) being particularly common [35-37]. The instrument used in this study is akin to the previous method in that it measures the movement of air bubbles in the capillary tube. However, it boasts a resolution of 0.196 nL, which is ten times more precise than that of the previous equipment [33]. Furthermore, unlike prior fluid filtration experiments that were confined to a specific point in time, the values obtained in this study were measured with sharp precision in real-time.

When measuring nanoleakage using fluid filtration equipment, it is important to start the measurement once the fluid flow has stabilized. In this study, we established the starting point for measurement at 300 seconds post specimen-instrument connection. This was done to allow ample time for the distilled water to saturate both the specimen's metal tube and the instrument's silicon tube, thereby obtaining a realistic nanoleakage value.

According to the results of this study, nanoleakage decreased significantly after 1 week compared to 24 hours across all experimental groups. Furthermore, there was no significant difference observed in nanoleakage between 1 week and 1 month. This suggests that the substantial reduction in nanoleakage can be attributed to the hardening expansion of the sealer 1 week after root canal filling, with stability observed thereafter.

When comparing nanoleakage at the 1-month mark, there was no significant difference among the groups, except for the experimental group in which the root canal was completely dried. In instances where the root canals were entirely dried, nanoleakage was consistently higher than in root canals dried using other methods, regardless of the time point. This suggests that the type and amount of residual moisture in the root canal do not impact the setting of the sealer and nanoleakage, provided the root canal is not completely dried. Therefore, in a clinical setting, when filling a root canal with a calcium silicate-based sealer, it is beneficial to prevent the root canal from becoming overly dry in order to achieve optimal treatment results.

In contrast, calcium silicate-based sealers are typically said to require moisture for setting. However, in this study, the sealer set even in completely dried root canals, thereby reducing nanoleakage. The first reason for the sealer's setting in a completely dried root canal could be the distilled water introduced into the specimen during nanoleakage measurement. This could include water introduced via the root apex and metal tube during storage, a process that involved covering with wet gauze. Additionally, even after the application of ethanol and storage at room temperature, the sealer might have cured due to residual moisture in the dentinal tubules. Therefore, a limitation of this study is that the moisture introduced into the metal tube during the experiment and storage process, as well as the moisture remaining in the dentinal tubules—which could not be completely eliminated despite efforts to dry the root canal—may have influenced the results of the study.

In previous studies comparing the quality of root canal filling according to root canal drying methods, EDTA and distilled water were used to remove as much residual material as possible from the root canal after its formation, regardless of the type of final irrigant solution used. However, in a typical clinical setting, NaOCl is generally the final irrigating solution present in the root canal. To better reflect this clinical reality, our study compared nanoleakage by categorizing the final irrigation solutions. According to our findings, when a small amount of moisture is left inside the root canal—achieved by drying the root canal using 2 paper points—there was no significant difference in nanoleakage between the groups using distilled water and NaOCl as the final irrigating solution after 1 month. In this study, we opted to use distilled water instead of saline to evaluate nanoleakage solely based on the condition of residual moisture. This is because physiological saline can hasten the setting reaction of calcium silicate-based sealers due to the ions present in the solution [38,39]. However, if future studies were to use physiological saline instead of distilled water, considering the potential for an accelerated setting process when physiological saline is used, it could lead to a more robust apical seal. This could, in turn, yield results related to an improved sealing ability.

The quality of root canal filling is evaluated based on 2 criteria: sealing ability and bond strength. An emphasis is often placed on the ability of the existing resin-based sealer to adhere to the root canal wall, using bond strength as a measure of the quality of the root canal filling. Neelakantan *et al.* [40] found a strong correlation between these 2 measures in a study involving root canals filled with resin-based sealers. However, they questioned the necessity for the root canal filling material to possess bond strength.

The bond strength test is both partial and destructive, as it only evaluates a specific area within the filled root canal. Conversely, the nanoleakage test, which assesses the sealing ability, examines the relationship between the entire root canal wall and the filling material. This test allows for repeated measurements without causing damage to the specimen. Given these experimental characteristics, and considering that the objective of root canal filling is to prevent bacterial entry by fully sealing the entire root system in 3 dimensions, it is posited that the evaluation of sealing ability through nanoleakage measurement is more beneficial for assessing the quality of root canal filling than the bond strength test.

Previous studies on the bond strength in relation to the moisture condition within the root canal have reported findings consistent with the current study. The experimental group with a completely dried root canal demonstrated the lowest bond strength when compared to the group where the root canal was left moist [19,20]. These studies suggest that excessive drying of the root canal may impede the penetration of the hydrophilic sealer into the dentinal tubules, thereby reducing bond strength [20]. The presence of moisture within the root canal is crucial for the hydration reaction between the sealer and residual moisture. This reaction results in the deposition of apatite minerals on the surface, facilitating a chemical bond between the sealer and dentin. This process ultimately leads to biocompatible characteristics [19].

In this study, the same clinician was involved in both specimen preparation and experimentation to ensure as much consistency in conditions as possible. However, due to the use of actual extracted human teeth, standardization of unique tooth characteristics, such as root canal size and anatomical shape, was not achievable. Furthermore, in a real root canal, factors such as dentinal tubular fluid and blood can influence the water conditions, but these were not accounted for in this experiment. Lastly, while this study investigated nanoleakage up to 1 month post root canal filling, further research is required to understand the longer-term trends.

CONCLUSIONS

In root canals filled with a calcium silicate-based sealer, nanoleakage according to the root canal drying method and the type of final irrigating solution was measured using NFMD at 24 hours, 1 week, and 1 month. The following conclusions were obtained. Completely dried root canals showed significantly higher nanoleakage at all time periods than root canals dried by other methods. In all groups, nanoleakage was significantly reduced and sealing ability was improved after 1 week compared to after 24 hours, and there was no significant difference in after 1 week and 1 month. The type of final irrigating solution did not affect nanoleakage.

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